

Research Protocol

Project Title: Family-centered oral health promotion for new parents and their infants: a randomized controlled trial

1. Study population and subject recruitment:

The study populations are the *new parents and their babies* by recruiting first-time expectant mothers and their husbands who have enrolled in the prenatal care program at the Maternal and Child Health Centers (MCHC) or Obstetrics & Gynecology Department (O&G) in public hospitals in Hong Kong.

The inclusion criteria are: i) First-time pregnancy; ii) of Chinese ethnicity; iii) ability to speak Cantonese/Mandarin and read traditional/simplified Chinese. The exclusion criteria are: i) pregnant women with any communication difficulties noted; ii) with informed consent not obtained.

After ethics approval from Department of Health and Hospital Authority, the first-time expectant mothers and their husbands will be recruited from the assigned MCHCs and O&G in the participated hospitals during their prenatal visit (around 12~20 weeks of the gestation period).

The staffs of the collaborated MCHCs and O&G will help informing the target group about the research and divert those interested to the recruitment counter after they have completed their antenatal visits.

We will be responsible for the recruitment, data collection process and the arrangement of the follow up appointments. The potential participants will be recruited into our study at the recruitment counter in the waiting area during their visits with minimal disturbance to the routine prenatal care to be given at the MCHCs and O&G and no advertisement is needed. All the details of the project will be explained and written informed consent will be obtained from the recruited subjects. The personal contact information (address, home and mobile phone numbers) of the recruited subjects will be obtained to facilitate the arrangement of all subsequent appointments.

2. Study design:

This study is a randomized controlled trial with parallel design. The recruited families will be randomly allocated to the test and control groups (Figure 1). Block randomization with a block size 4 will be adopted; the random numbers sequence will be generated by the EXCEL computer software. Opaque sealed envelopes will be used to conceal the allocation sequence until the interventions are assigned. The ICH-GCP and CONSORT statement will be strictly followed.

Test group - Oral health education (OHE) program:

A baseline dental examination and questionnaire survey for the expectant mothers and their husbands will be conducted around 12~20 weeks of the gestation period after obtaining the participants' consent. The sites of data collection would be as follows *with priorities*:

For MCHCs:

- 1) The School Dental Clinics (DC) located in the same building or nearby *or*

- 2) Prince Philip Dental Hospital (PPDH, the teaching hospital of Faculty of Dentistry, HKU where the primary investigators initiated this project) *or*
- 3) Home visit

For O&Gs:

- 1) An appropriate space in the O&G Department (maybe a separate room, a quiet corner, etc.) *or*
- 2) Prince Philip Dental Hospital (PPDH, the teaching hospital of Faculty of Dentistry, HKU where the primary investigators initiated this project) *or*
- 3) Home visit

OHE (Part I): Personal oral hygiene instruction (OHI)

Pamphlets for adults' and pregnant women's oral health care will be distributed and individual personal hygiene instruction will be given by dentists or trained dental auxiliary staff to both the expectant mothers and their husbands after the baseline dental examination (conducted by dentists not involve in the OHI) and questionnaire survey aiming to establish their self-efficacy in oral health care for themselves.

Contents of OHE (Part I) are listed below:

- 1) Distributing the pamphlet for adults' oral health care: 清潔牙齒，點只刷牙咁簡單? Cleaning your teeth, just by toothbrushing? (published by the Oral Health Education Unit, Department of Health)
- 2) Distributing the pamphlet for pregnant women's oral health care: 準媽媽的口腔常識 Oral Health for the Expectant Mother (published by the Oral Health Education Unit, Department of Health)
- 3) Explaining in details about the contents of the pamphlets
- 4) Demonstration of the methods of tooth brushing and dental flossing
- 5) Personal oral health instruction and Q&A

OHE (Part II): OHI + information on infancy oral health

Around the 32nd week of the gestation period, reinforcement of OHI will be delivered to both the expectant mothers and their husbands after the dental examination and questionnaire survey. Printed information in a booklet on the tooth formation and eruption, proper feeding and dietary habits for their new born babies will be given and explain to them during this visit to prepare them for the tooth eruption or teething problem that they may encounter and to establish proper feeding dietary habits for their new babies. The site of data collection this time would be better kept consistent with the baseline data collection, i.e. School Dental Clinics located in the same building of the MCHCs or nearby, O&G clinics, PPDH or home visits. Nevertheless, if the participants might not stay with the same MCHC or O&G for all antenatal visits (including those who may register their children with another MCHC after delivery), they will be followed up in another site: e.g. another School Dental Clinic or O&G clinic which is also one of our recruitment sites, PPDH or home visit. All the participants will be contacted through phone calls for arranging the appointment.

Contents of OHE (Part II) are listed below:

- 1) Distributing the booklet for babies' oral health care: 幼兒口腔護理全接觸 Oral Health Care for your Children (published by the Oral Health Education Unit, Department of Health)
- 2) Explaining in details about the following contents of the booklet: tooth formation and eruption, proper feeding habits for babies 0-6 months old, the method of cleaning babies' mouth.

- 3) Reinforcement of adults' oral health care
- 4) Q&A

OHE (Part III): OHI + infant oral cavity cleaning & toothbrushing

After the babies having been born and reaches to 6-month-old, reinforcement of OHI will be delivered to the new parents. Reinforcement of maintenance of proper feeding and dietary habits for their babies and demonstration to the new parents how to clean the oral cavity cleaning and perform toothbrushing of their babies will be delivered. No dental examination and questionnaire survey will be conducted at this visit. The site to deliver OHE this time will be in the School Dental Clinics in the same building of the MCHCs or nearby where the participants will bring back their babies for vaccination, PPDH or home visit. All the participants will be contacted through phone calls for arranging the appointment.

Contents of OHE (Part III) are listed below:

- 1) Explaining in details about the following contents of the booklet 幼兒口腔護理全接觸: discomfort during tooth eruption, proper feeding habits for babies 6-12 months old
- 2) Demonstration of how to clean the oral cavity and perform toothbrushing for the babies
- 3) Reinforcement of adults' oral health care
- 4) Q&A

OHE (Part IV): OHI + infant toothbrushing

A home visit to reinforce the toothbrushing techniques for their infants that was taught around the 6-month visit will be arranged when the babies reached 1-year-old, dental examination and questionnaire survey on the parents and their babies will be conducted and reinforcement of OHI will be delivered.

Contents of OHE (Part IV) are listed below:

- 1) Explaining in details about the following contents of the booklet 幼兒口腔護理全接觸: proper feeding habits for babies 1 year old or above, regular dental checkup and early childhood caries
- 2) Reinforcement of toothbrushing technique for babies
- 3) Q&A

OHE (Part V): OHI + infant toothbrushing (reinforcement)

Home visit of another reinforcement of the toothbrushing techniques for their infants will be arranged when the babies reached 2-years-old, dental examination and questionnaire survey on the parents and their babies will be conducted and reinforcement of OHI will be delivered. The content of OHE (Part V) is the same as OHE (Part IV).

An additional home visits will be arranged when the babies reached 3-year-old for dental examination and questionnaire survey on the parents.

Control group:

For the control group, two dental examinations and questionnaire surveys will be arranged for the expectant mothers and their husbands also around 12~20 weeks of the gestation period and around 32nd –week. The sites of data collection will be the same as that of the TEST group (as indicated on P.2). Pamphlets for adults' and pregnant women's oral health care will be distributed around 12~20 weeks and information on the babies' oral health care will be distributed around the 32nd week (same pamphlets as the Test group). Three home visits will

be arranged to conduct dental examinations and questionnaire surveys of the new parents and their babies when the babies reached 1-, 2- and 3-year-old respectively.

The pamphlet named “準媽媽的口腔常識 Oral Health for the Expectant Mother” has been distributed to all the pregnant women as part of the standard prenatal care programme to be given at MCHC. And the pamphlet named “幼兒口腔護理全接觸 Oral Health Care for your Children” has been distributed to parents as a chapter in the Happy Parenting Book 3 to be given at MCHC when the infants reached 1 month of age.

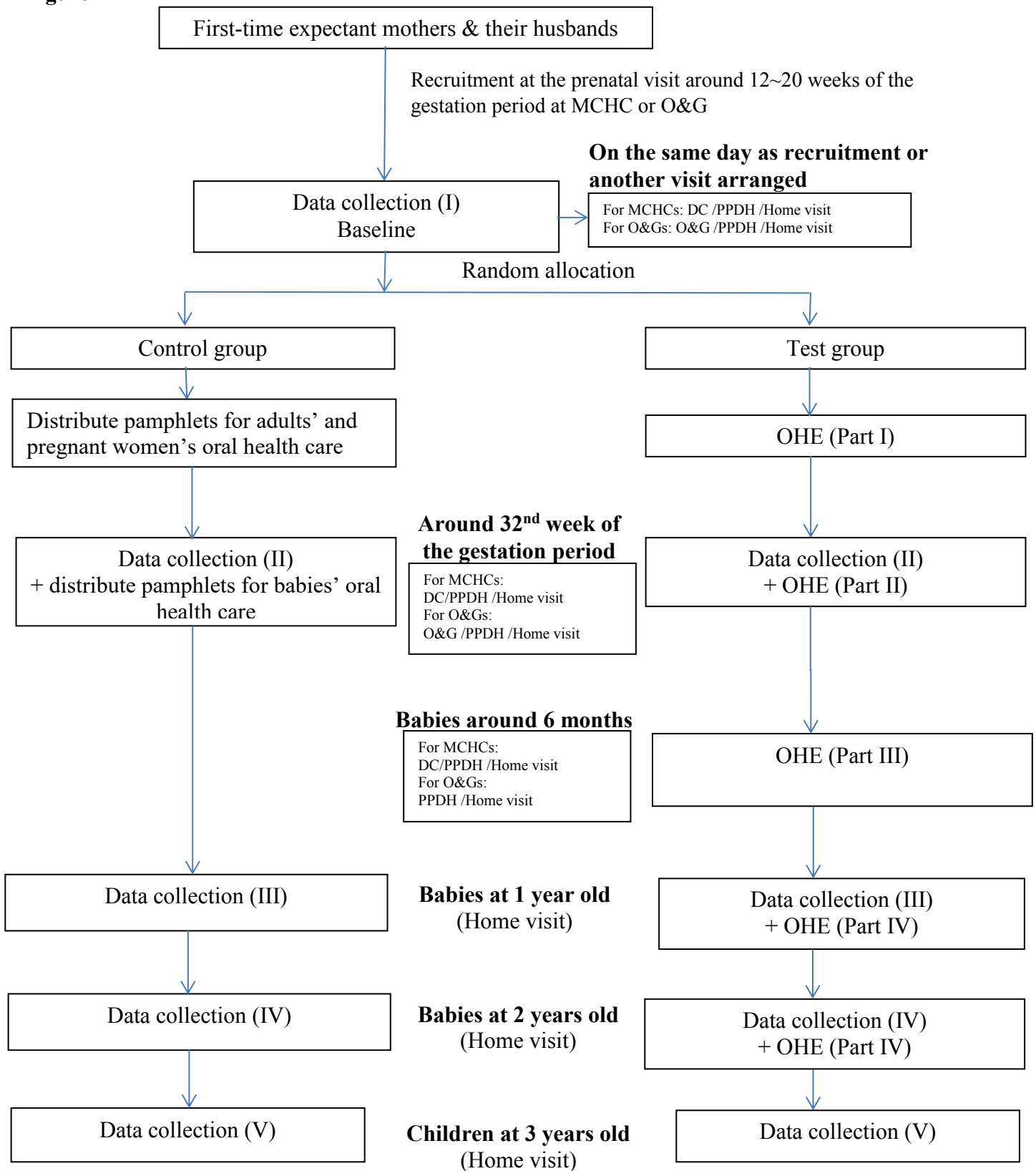
3. Sample size calculation:

The main primary outcome of this study is the prevalence of early childhood caries (ECC) for children at 3-year-old. Based on the reported prevalence of ECC for 3-year-old children (31%), we anticipate that the prevalence of ECC for children at 3-year-old in the control group in this study will be similar (i.e. 31%). A 40% reduction in the prevalence of ECC in the test group compared to the control group (i.e. to 19%) is considered to be of clinical significance.

The other primary outcome is the proportion of parents who will brush their infants' teeth regularly at 1-year-old. Based on data from our ongoing project, about 15% of the parents brushed their infants' teeth at 1-year-old. We anticipate that the situation will be similar in the control group in this study and there will be at least 30% of the parents in the test group will brush their infants' teeth.

Result of a sample size calculation, based on a 5% statistical significance level and an 80% power, shows that a minimum of 204 babies in each group are required for the ECC outcome and 121 babies for the toothbrushing outcome. Since we distribute oral health education materials to the control group as a standard care, we hope the control group can get some benefit as well. If we assume a minimal improvement in the control group with a 5% decrease in ECC prevalence (i.e. from 31% to 29%), and keep a 40% reduction in the prevalence of ECC in the test group compared to the control group (i.e. 17%), the required sample size will be 192 for each group.

Taking the larger required sample size and to allow for loss of power due to drop-out over the study period, the initial sample size should be around 30% larger, i.e. 292 families (expectant mothers, expectant fathers and their infants) are to be recruited in each group at the beginning of the study. Thus, the total initial sample size will be 584 families (expectant mothers, expectant fathers and their infants).

Figure 1

4. Data collection:

Comprehensive data will be collected from the expectant mothers, their husbands and the infants in the test and control groups through dental examination, biological testing and questionnaire survey at baseline, 32nd week of the gestation period, 1-, 2- and 3-year-old (Table 1).

4.1 Dental examination:-

Methods, equipment, and indices as recommended by the World Health Organization (WHO) for conducting oral health surveys will be employed. An intra-oral LED light, disposable mouth mirrors and dental probes recommended by the WHO will be used. Some standard measurements of the soft tissues of certain teeth would be made and there is a little bit discomfort however the procedures do not hurt and will only take a few minutes. No special equipment needed and no x-ray will be taken. There is no hazards associated with the pregnant women and babies. This study will comprehensively assess the clinical oral health status of the study subjects using trained, experienced, and calibrated examiners.

The examiners (dentists not involved in the oral health instruction or education) will be blinded to the group allocation of the participating families.

Dental caries (DC) for adults

The overall dental caries (tooth decay) experience of the subjects will be recorded by counting the number of teeth (T) that are decayed, missing due to caries, and filled for the calculation of the DMFT. Active caries into dentine (DT) will be diagnosed at the cavitation level through careful visual inspection. A ball-ended CPI probe will be used when necessary to remove food debris and plaque that obscure visual inspection and to confirm the presence of a cavity.

Dental caries (DC) for babies & children:

The tooth status of the erupted primary teeth will be assessed by careful visual inspection using a mouth-mirror attached to an intraoral LED light. A ball-ended probe CPI will be used only when necessary to remove any remaining plaque and debris that obscure inspection and to check for minor cavitation. Diagnostic for ECC will be based on modified version of the International Caries Detection and Assessment System (ICDAS) criteria in epidemiological surveys.

Oral hygiene status (VPI) for adults

Oral hygiene status will be charted by using the Visible Plaque Index. The presence or absence of plaque on the buccal and lingual surfaces of six index teeth (16, 21, 24, 36, 41, 44) will be recorded.

Oral hygiene status (VPI) for children

Oral hygiene status will be charted by using the Visible Plaque Index. The presence or absence of plaque on the buccal and lingual surfaces of all primary teeth will be recorded.

Periodontal health (CPI) for adults:

The periodontal status of the upper and lower central incisors, and the first and second molars will be examined with the aid of a graduated periodontal probe. The greatest periodontal probing depth, presence of calculus, and bleeding on probing will be recorded for each tooth examined. From the parameters recorded, indices for measuring periodontal status, including the community periodontal index (CPI) can be calculated. The largest extent of loss of periodontal attachment in the index teeth examined will be recorded.

4.2 Biological testing:-

MS quantitation:

Unstimulated saliva and plaque samples will be collected. Unstimulated saliva will be collected using dropper for babies and spitting out into sterilized tube by mothers. Plaque samples will be collected from the surfaces of the anterior teeth of the mothers and the babies using sterile cotton tips, and will be processed within one day of collection. Tips will be vortexed in phosphate buffered saline (PBS) buffer, then centrifuged to collect the bacterial cells. After washing (PBS, 1 ml), cells will be lysed according to the procedure of Paster *et al.* (2001), to provide DNA template for quantitative analysis of *S. mutans* levels using 'species-specific' Taqman (real-time) quantitative polymerase chain reaction (Q-RT-PCR) as has been used in our previous study (Gao *et al.*, 2012). Briefly, plaque samples will be washed in PBS buffer, then resuspended in 50µl of lysis buffer [50mM Tris-HCl (pH 7.6), 1 mM EDTA, 0.5% Tween 20, and proteinase K (10 µg)], heated at 55°C for 2 hours, then at 95°C for 5 minutes. DNA extracted from two *S. mutans* ATCC cultures (ATCC 35668 and ATCC 700610) will be used as positive controls. Q-RT-PCR will be performed using the ABI PRISM 7900HT sequence detection system using species-specific Taqman probes (Applied Biosystems, Carlsbad, CA, USA). This Taqman assay has been further validated to quantify *S. mutans* counts over the range 10^3 – 10^9 cells, and this standard will be included in each Q-RT-PCR run, used to quantify the bacterial counts in the plaque samples. All samples will be analyzed in duplicate. Each PCR will be performed in a total volume of 20 µL consisting 10 µL of Taqman Gene expression master mix, 1 µL of forward and reverse primers, 1 µL of Taqman probe, 1 µL of template DNA and appropriate amount of sterilized DNase–RNase-free water. The cycling conditions will be 50°C for 2 min, 95°C for 10 min, 40 cycles at 95°C for 15 s, and 60°C for 1 min.

4.3 Questionnaire survey:-

Before each clinical examination, the expectant mothers and their husbands will be asked to complete a questionnaire that assesses their oral health (OH) knowledge, attitude and behaviours and oral health related quality of life (OHQoL).

OH knowledge: Knowledge of the causes and prevention of dental caries and periodontal disease. Knowledge of oral health care for babies.

OH attitude: Attitude toward the importance of oral health, the importance of retaining natural teeth, dental service utilization, and dental health beliefs for adults and babies.

OH behaviours: Oral health behaviour relating to oral hygiene practices, use of fluoride products, and dental attendance patterns. In addition, assessment of dental care coverage and level of dental care received. The new parents will also be asked about the feeding, dietary and toothbrushing habits of their babies at 1, 2 and 3 years old.

OHQoL: Using the Chinese version of short form of Oral Health Impact Profile (OHIP-14) to assess how often the participants experience the impact of problems with teeth, mouth or dentures on daily life.

Table 1

			Dental Examination			Biological testing	Questionnaire Survey
	Test	Control	DC	VPI	CPI		
Baseline data collection (I)	√	√					
Expectant mother			√	√	√	√	√
Husbands			√	√	√		√
Data collection (II) 32nd week	√	√					
Expectant mother				√	√	√	√
Husbands				√	√		√
Data collection (III) babies 1 year	√	√					
Mother			√	√	√	√	√
Father			√	√	√		√
Babies						√	
Data collection (IV) babies 2 years	√	√					
Mother			√	√	√	√	√
Father			√	√	√		√
Babies			√	√		√	
Data collection (V) babies 3 years	√	√					
Mother			√	√	√	√	√
Father			√	√	√		√
Children			√	√		√	

5. Data analysis:

The collected data will be entered into a computer and analyzed by using the statistical software SPSS for Windows version 20.0.

Intention to treat analysis will be carried out. The primary outcomes of the OHE program are the proportion of parents brushing their infants' teeth at 1 year old and the prevalence of ECC in the study children at 3 years old. Other outcome variables include: the mothers' and the babies' MS counts; the babies' feeding and dietary habits; the mothers' and fathers' oral hygiene status, dental caries, and periodontal condition over the study period.

The effectiveness of the OHE will be evaluated by comparing the differences in the outcome variables between the test and control groups. Chi-square tests and two-sample t-tests will be performed to test the differences in the proportions of the categorical outcome variables and the means of the continuous outcome variables.

The association between the mothers' and the infant's MS counts will be investigated by Spearman's rank correlation coefficient. The effects of MS counts of the mothers and infants; toothbrushing, feeding and dietary habits of the infants on the development of ECC, together with a number of possible confounding factors, such as, the socio-economic status, oral health status, knowledge, attitudes and behaviours of the parents will be investigated by multiple logistic regression and structural equation modeling. The level of statistical significance for all tests will be set at 0.05.

References

1. Gao XL, Seneviratne CJ, Lo ECM, Chu CH, Samaranayake LP (2012). Novel and conventional assays in determining abundance of *Streptococcus mutans* in saliva. *International Journal of Paediatric Dentistry* 22(5):363-368.
2. Paster BJ, Boches SK, Galvin JL, Ericson RE, Lau CN, Levanos VA, Sahasrabudhe A, Dewhirst FE (2001). Bacterial diversity in human subgingival plaque. *Journal of Bacteriology* 183: 3770-3783.